

## Short Communication

# Characterizing Imiquimod-Induced Psoriasis-like Dermatitis in BALB/c Mouse: Application in Dermatology Research

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## Keywords

• Imiquimod model; Chronology; Histological features; Ki67; Experimental design

## Abstract

The imiquimod (IMQ)-induced psoriasis-like dermatitis mouse model is a powerful tool in psoriasis research. The model, however, has not been fully characterized for its optimal application in research. Our study aims to define the chronology of an IMQ-induced inflammatory skin reaction in BALB/c mouse with the goal of optimizing the induction protocol for future psoriasis research. We applied IMQ 5% cream on the skin of BALB/c mouse. Subsequently, the skin was harvested and processed for H&E staining and Ki67 immunostaining. Once daily topical application of IMQ on BALB/c mouse induced psoriasis-like dermatitis clinically and histologically beginning on day 2 and peaking on days 4 and 5. Correspondingly, the topical application also induced a hyperproliferative epidermis in a similar fashion. These changes were acute in nature and not sustainable beyond day 5, despite continued application of IMQ. However, once the skin was allowed to recover completely back to baseline, then IMQ application was able to re-induce psoriasis-like dermatitis. Understanding how best to manipulate the model to meet the needs of an individual experimental design may help researchers to avoid unnecessary optimization processes and to obtain robust outcomes.

## ABBREVIATIONS

IMQ: Imiquimod; AKs: Actinic Keratoses; sBCCs: Superficial Basal Cell Carcinomas; PDCs: Plasmacytoid Dendritic Cells

## INTRODUCTION

Imiquimod (IMQ) stimulates the innate immunity by activating toll-like receptor 7. This activation leads to a cascade of intracellular signaling events that results in the secretion of cytokines, such as IFN $\alpha$ , IL-6, and TNF $\alpha$ . By activating the immune system, IMQ exerts its unique antitumoral activity and thus is currently FDA-approved for the treatment of actinic keratoses (AKs), superficial basal cell carcinomas (sBCCs), and genital warts. With its increased use, there have been multiple reports of psoriasis-like reaction at the site of application. Wu et al., was the first to report a case of a patient with pre-existing psoriasis, who developed local psoriasiform dermatitis from IMQ use for the treatment of sBCCs [1]. Subsequently, other groups reported cases of generalized psoriasis in patients treated with IMQ for sBCCs and AKs [2,3].

The hallmark of IMQ-induced psoriasis is the infiltration of plasmacytoid dendritic cells (PDCs) in the treated human skin with subsequent production of IFN $\alpha$  [4]. Indeed, both systemic and intralesional IFN $\alpha$  have been demonstrated to induce and/or exacerbate psoriasis in humans [5-12]. Similarly, topical application of IMQ in mice has been shown to bring about infiltration of PDCs and induce epidermal hyperplasia [13]. Furthermore, van der Fits et al., have demonstrated that the

IMQ-induced dermatitis in mice does resemble human psoriasis clinically and histologically, and the skin inflammation is critically dependent on IL-23 and IL-27 [14]. Since the introduction of the IMQ-induced psoriasis-like dermatitis mouse model, multiple groups have used this model to study the pathogenesis of psoriasis and to assess the therapeutic effects of potential drug targets for psoriasis.

## MATERIALS AND METHODS

## Institutional Review Board

The animal study protocol was approved by the Institutional Animal Care and Use Committee at Boston University under the protocol number, AN-15609.

## Topical Imiquimod Induction

BALB/c female mice between the ages of 6-7 weeks were purchased from The Jackson Laboratory (Bar Harbor, Maine). The lower back of each mouse was shaved to an approximately 1 cm<sup>2</sup> area. To the shaved area, a 2.5 g equivalent of active IMQ 5% cream (Henry Schein) was applied evenly as a thin film once daily for 5 days. The mice were then split into three groups after the initial 5 day IMQ induction: 1) continued IMQ induction once daily from day 6 to day 10, 2) continued IMQ on alternate days from day 6 to day 10, and 3) left untreated for IMQ re-induction until day 20. Each day, clinical photographs were obtained to track the inflammatory response. Simultaneously, the skin was harvested and processed for standard paraffin embedding, followed by

H&E staining (performed by the Skin Pathology Laboratory at Boston University). The experiments were repeated as biologic duplicates, two completely independent experiments (27 mice for each experiment) to confirm the findings.

### Immunohistochemistry from Paraffin-embedded Tissue

Deparaffinization, rehydration, antigen unmasking, and immunostaining were performed according to the Abcam protocols with the following exceptions. For unmasking step, tissue sections were incubated with sodium citrate buffer at sub-boiling temperature for 30 minutes. To block endogenous peroxidase activity and immunoglobulin cross-reactivity, the sections were incubated with 3% hydrogen peroxide and Animal-Free Blocking Solution (Abcam #15019) at room temperature for 10 minutes and 1 hour, respectively. For detection step, the sections were incubated at room temperature for 1.5 hours with anti-Ki67 antibody (Abcam #15580) diluted in 1:400 in Signal Stain Antibody Diluent (Cell Signaling Technology #8112), then incubated with 1-2 drops of Signal Stain Boost Detection Reagent (Cell Signaling Technology #8114) at room temperature for 30 minutes, followed by 2 drops of Signal Stain DAB substrate (Cell Signaling Technology #8059) at room temperature for 5-10 minutes.

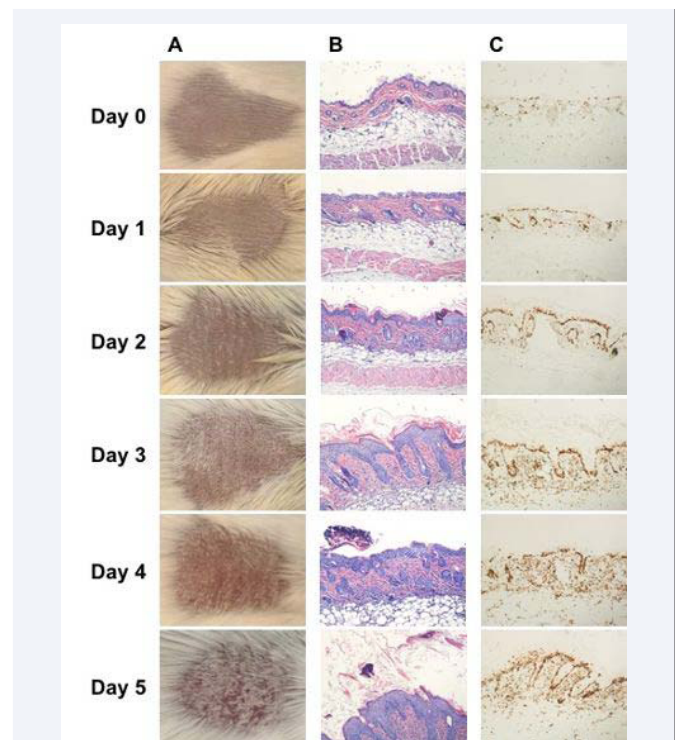
## RESULTS

### Topical application of IMQ induces psoriasis-like histologic changes and hyperproliferative epidermis.

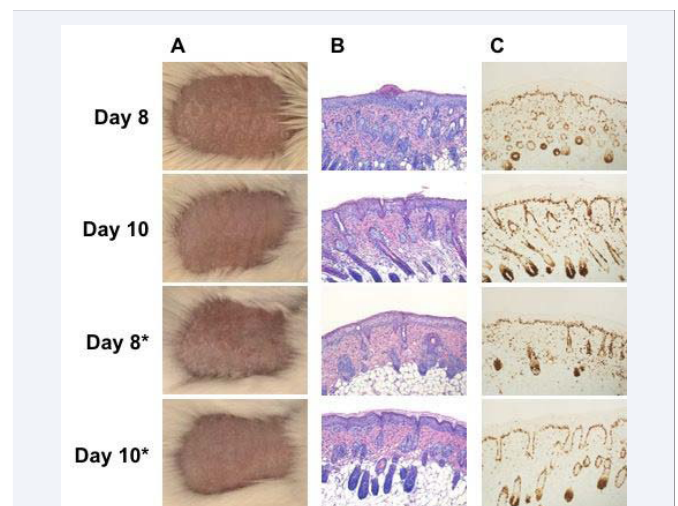
We examined the effects of daily topical IMQ application on the skin of BALB/c mice. IMQ was applied once daily for 5 days to the shaved backs of BALB/c mice. Clinically (Figure 1, Column A), the skin demonstrated increasing erythema, thickness, and scaling. On H&E staining (Figure 1, Column B), the histology demonstrated increasing acanthosis, parakeratosis, neutrophils in the stratum corneum, and inflammatory infiltrates in the dermis. On Ki67 staining (Figure 1, Column C), the epidermis demonstrated an increasing number of proliferating keratinocytes. Clinically and histologically, the corresponding effects peaked in day 4 and 5 of the topical application.

### Continued topical application of IMQ does not sustain psoriasis-like histologic changes and hyperproliferative epidermis

Next, we assessed the sustainability of psoriasis-like histologic changes and the hyperproliferative epidermis. After first 5 days, IMQ application was continued once daily or once every other day for additional 5 days (labeled as day 6 to day 10). Despite continued IMQ application, the skin demonstrated decreased erythema and thickness with loss of scales (Figure 2, Column A). On H&E staining (Figure 2, Column B), the histology demonstrated decrease in acanthosis, conversion from parakeratosis to orthokeratosis, and the loss of neutrophils in the stratum corneum. On Ki67 staining (Figure 2, Column C), the epidermis demonstrated a decreasing number of proliferating keratinocytes. We also quantitated the number of Ki67 staining cells in the epidermis from day 0 to day 10, as demonstrated in Figure 3.



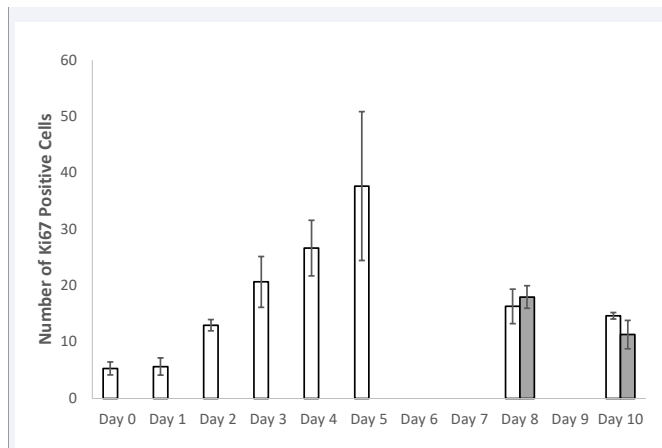
**Figure 1** Once daily topical application of IMQ induces psoriasis-like dermatitis and hyperproliferative epidermis in BALB/c mouse. (A) Representative clinical photo. (B) Representative H&E stain. (C) Representative Ki67 stain.



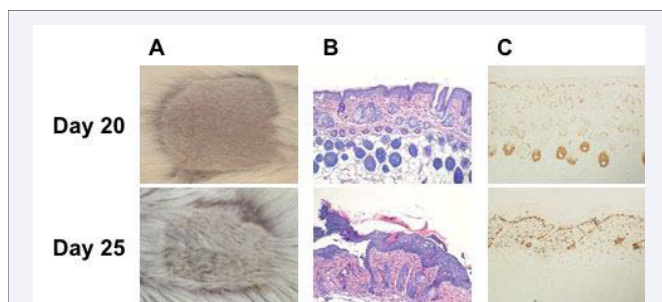
**Figure 2** Continued topical application of IMQ in BALB/c mouse does not sustain psoriasis-like dermatitis. After initial IMQ-induction for 5 days, IMQ application was continued once daily or alternate day (\*) from day 6 to day 10. (A) Representative clinical photo. (B) Representative H&E stain. (C) Representative Ki67 stain.

### After complete recovery, IMQ can re-induce psoriasis-like histologic changes and hyperproliferative epidermis

Upon cessation of IMQ application, the psoriasis-like dermatitis resolved on its own. Mice were treated topically with



**Figure 3** The number of Ki67 positive cells in the epidermis decreases despite continued topical application of IMQ in BALB/c mouse. IMQ application during the first 5 days increases the number of proliferating cells in the epidermis. Continued application beyond day 5 does not sustain the number of proliferating cells in the epidermis. Using Figures 1C and 2C, three separate areas of equal size across the epidermis were gated and Ki67 positive cells were counted and averaged as shown above with error bars representing standard deviations. Empty bars represent daily application and gray bars represent alternate day application.



**Figure 4** After complete recovery from initial induction, IMQ can re-induce psoriasis-like dermatitis and hyperproliferative epidermis in BALB/c mouse. (A) Representative clinical photo. (B) Representative H&E stain. (C) Representative Ki67 stain.

IMQ once daily for 5 days, then left untreated for following 15 days. On day 20 (Figure 4, first row), the skin returned to baseline clinically and on H&E stain. The epidermis also demonstrated the baseline Ki67 staining. Subsequently, the skin was able to undergo re-induction with IMQ in a similar fashion. After once daily topical application of IMQ for 5 days (Figure 4, second row), the skin demonstrated the psoriasis-like dermatitis clinically and on H&E stain. The epidermis also demonstrated an increase in Ki67 staining.

## DISCUSSION

Our study aimed to characterize the IMQ-induced psoriasis-like dermatitis mouse model for the purposes of streamlining future psoriasis research in dermatology. A better understanding of the chronology with respect to the onset, peak, resolution, and/or re-induction of the IMQ-induced reaction can greatly assist researchers in their experimental designs. Based on our findings, IMQ-induced psoriasis-like dermatitis

begins on day 2 and reaches its peak intensity on days 4 and 5 both clinically and histologically. In addition, IMQ-induction increases the number of proliferating keratinocytes in the epidermis correspondingly. Beyond day 5, psoriasis-like dermatitis begins to resolve despite continued daily or every other day application of IMQ. Starting on day 8, the skin is significantly thinner and less erythematous with loss of scales. Histologically, the features of psoriasis-like dermatitis are significantly attenuated with loss of parakeratosis and acanthosis. On Ki67 stain, the proliferating keratinocytes in the epidermis starts to decrease in number. After complete recovery back to baseline clinically and histologically, IMQ re-induction can be achieved within 5 days in a similar fashion. Taken together, our study demonstrates that IMQ-induction of psoriasis-like dermatitis is short-lived and does not sustain beyond day 5. Understanding the acute nature of this inflammation allows researchers to target the optimal days for their experiments. When evaluating potential therapeutic effects of topical agents, it may be advisable to assess the degree of IMQ-induction inhibition. In contrast, it may not be ideal to test the degree of recovery after IMQ-induction, as recovery begins regardless starting with day 5. If necessary, the skin can be re-induced with IMQ after a complete recovery from the first reaction. It appears that there is no significant immune memory that changes the characteristics of a second induction as compared to the first.

## CONCLUSION

IMQ-induced psoriasis-like dermatitis is a convenient, cost-effective, and simple mouse model. However, it is important to consider its limitations. Psoriasis is a chronic inflammatory skin disorder. In contrast, IMQ-induced psoriasis-like dermatitis is an acute inflammatory response that is not sustainable. As such, researchers using this mouse model to test therapeutic compounds should carefully design their experiments to take advantage of its unique features.

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